Biomarkers of Rejection and Tolerance in Liver Transplantation

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Northwestern University
Disclosures

Transplant Genomics, Inc (advisor, stockholder)
Novartis (speaker, research funding)
Learning Objectives

Review immunological issues in liver transplant recipients in the current era

Discuss which clinical situations biomarkers could advance diagnosis and management of rejection in liver transplantation

Review the current data demonstrating the use of biomarkers in liver transplant recipients
Long term complications in LTR all linked in part to IS therapy

- Malignancy – nearly half
- Cardiovascular Disease – most
- Renal dysfunction - most
- Infection – most
- Drug side effects - most
- Cost – all

- Immunological graft failure: believed to be uncommon
Why worry about rejection in LT recipients?

Probability of Posttransplant Mortality and Allograft Failure Over Time, by Time of First Posttransplant BPAR

Current Approach to Monitoring Level of Immunosuppression in LTR

• Clinical History
  – Age
  – History of rejection(s) vs. over-immunosuppression
  – Immune vs. Non-Immune Disease
  – Viral vs. Non-Viral Disease - no longer

• Arbitrary trough levels
  – Poor correlation with degree of immunosuppression
  – Borrowed from renal transplantation
Holy Grail

**Traditional**

Tolerance (Withdrawal of IS therapy with normal graft function)

**Other/Alternative**

Biomarker predicting rejection or other complications guiding IS modifications (augment vs. minimize)
“…..omics” for **Biomarker Discovery**

- Need to associate a biomarker “signature” with a “phenotype”
  - rejection, tolerance, renal disease, response to therapy

- Need to decide which compartment is most relevant (blood, urine, graft..) and cell vs. plasma vs. parenchyma

- Validation of an exploratory set is key
Practical Considerations in Transplantation

- **Diagnostic biomarkers**: can we make a diagnosis with a less invasive modality with equal or higher sensitivity/specificity?
  - The biomarker may be no better and more $$ than a simple serum marker (ALT)

- **Predictive biomarkers**: can we predict biologic behavior?
  - development or progression of a condition
  - response to an intervention

- **Retrospective longitudinal studies**: can we use existing patients and biorepositories to inform and validate biomarker discovery?

- **Prospective longitudinal studies**: can we use future patients to best validate biomarker discovery?
Immunosuppression Modifications Based on an Immune Response Assay: Results of a Randomized, Controlled Trial

Matteo Ravaioli, MD, Flavia Neri, MD, Tatiana Lazzerotto, MD, PhD, Valentino Rosa Bertuzzo, MD, Paolo Di Gioia, MD, PhD, Giacomo Stacchini, MD, Maria Cristina Morelli, MD, Giorgio Ercolani, MD, PhD, Matteo Cescon, MD, PhD, Angela Chereghini, PhD, Massimo Del Gaudio, MD, PhD, Alessandro Cucchetti, MD, PhD, and Antonio D. Pinna, MD, PhD

Ravaioli et al. Transplantation 2015
### TABLE 2
Comparison of outcomes at 12 months of follow-up

<table>
<thead>
<tr>
<th></th>
<th>All patients (N = 202)</th>
<th>Intervention group (n = 100)</th>
<th>Control group (n = 102)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient survival, %</td>
<td>170 (84.2)</td>
<td>89 (89.0)</td>
<td>80 (78.4)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Event-free recipients, %</td>
<td>83 (58.9)</td>
<td>41 (41.0)</td>
<td>42 (41.2)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Infectious episodes &gt;14 d</td>
<td>98 (48.5)</td>
<td>42 (42.0)</td>
<td>56 (54.9)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>after transplantation, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute rejections, %</td>
<td>33 (16.3)</td>
<td>19 (19.0)</td>
<td>14 (13.7)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Recipients with &gt;3 infection, %</td>
<td>21 (10.4)</td>
<td>11 (11.0)</td>
<td>10 (9.8)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Bacterial infections, %</td>
<td>77 (57.1)</td>
<td>32 (32.0)</td>
<td>47 (46.1)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Fungal infections, %</td>
<td>13 (6.8)</td>
<td>2 (2.0)</td>
<td>11 (10.8)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Viral infections, %</td>
<td>45 (33.3)</td>
<td>22 (22.0)</td>
<td>23 (22.5)</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>All patients, MELD &gt; 20 (N = 99)</th>
<th>Intervention group, MELD &gt;20 (n = 44)</th>
<th>Control group, MELD &gt;20 (n = 55)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient survival, %</td>
<td>81 (81.8)</td>
<td>41 (93.2)</td>
<td>40 (72.7)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Infectious episodes &gt;14 d</td>
<td>60 (60.6)</td>
<td>22 (50.0)</td>
<td>38 (69.1)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

*Cause of 11 deaths: multiple organ failure (n = 2, 18%), infections (n = 3, 27%), recurrent HCV hepatitis (n = 1, 9.1%), surgical complication (n = 2, 18%), and tumor-related causes (n = 3, 27%).

*Cause of 22 deaths: multiple organ failure (n = 7, 32%), infections (n = 8, 36%), recurrent HCV hepatitis (n = 2, 9.1%), technical reasons (n = 2, 9.1%), and tumor-related causes (n = 3; 14%). MELD scores were measured the day before liver transplantation.

Ravaioli et al. Transplantation 2015
Ravaioli et al. Transplantation 2015
### LT Rejection Biomarkers

<table>
<thead>
<tr>
<th>Author</th>
<th>Phenotype</th>
<th>Source</th>
<th>Biomarker Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evans¹</td>
<td>CR</td>
<td>Recipient DNA</td>
<td>HLA-DR3, TNF-2</td>
</tr>
<tr>
<td>Gomez-Mateo²</td>
<td>AR</td>
<td>Recipient DNA</td>
<td>TGFβ-1 protective</td>
</tr>
<tr>
<td>Moya-Quiles³</td>
<td>AR</td>
<td>Recipient DNA</td>
<td>Recip HLA-Cw*07 protective</td>
</tr>
<tr>
<td>Sindhi⁴</td>
<td>AR</td>
<td>Recipient DNA</td>
<td>rs9296068 SNP</td>
</tr>
<tr>
<td>Hanvesakul⁵</td>
<td>Graft function</td>
<td>Donor DNA</td>
<td>Donor HLA-C protective</td>
</tr>
<tr>
<td>Massoud⁶</td>
<td>AR</td>
<td>Recipient serum proteins</td>
<td>C4, C1q</td>
</tr>
<tr>
<td>Li⁷</td>
<td>AR</td>
<td>Recipient DNA and mRNA</td>
<td>CCL3L1 gene CNV</td>
</tr>
<tr>
<td>Karimi⁸</td>
<td>AR</td>
<td>Recipient DNA</td>
<td>IL-6, IFN-γ</td>
</tr>
<tr>
<td>Fan⁹</td>
<td>AR</td>
<td>Recipient PBL</td>
<td>Th17 cells (CD4+, IL17+)</td>
</tr>
<tr>
<td>Farid¹⁰</td>
<td>AR and injury</td>
<td>Recipient serum and biopsy</td>
<td>Hepatocyte-derived miRNA (122, 148, 194)</td>
</tr>
<tr>
<td>Joshi¹¹</td>
<td>AR</td>
<td>miRNA (graft, sera)</td>
<td>miRNA 146, 19a, 20a, let-7e</td>
</tr>
<tr>
<td>Kamei¹²</td>
<td>AR</td>
<td>Donor and recipient DNA</td>
<td>GSTT1 genotype (d/r mismatch)</td>
</tr>
<tr>
<td>Shaked¹³</td>
<td>AR</td>
<td>miRNA</td>
<td>Can predict AR</td>
</tr>
<tr>
<td>Toby¹⁴</td>
<td>AR</td>
<td>Recipient PBMC proteoforms</td>
<td>Can predict AR and ADNR</td>
</tr>
</tbody>
</table>

Biopsy mRNA: AR vs. HCV-R vs. Mixed AR/HCV-R
An Ectopically Expressed Serum miRNA Signature Prognostic and Diagnostic of Liver Allograft Rejection

Proteoforms of Acute Rejection in LT

Toby TK et al. *Am J Transplant*. 2017
CTOT-14:
Prospective sample collection from the time of LT in 202 recipients (7 centers)

Main objective:
Diagnostic and predictive genomic signatures of AR and CKD in LTR
CTOT-14 Primary Objective: AR Diagnosis (AR vs. TX)

<table>
<thead>
<tr>
<th></th>
<th>AUC</th>
<th>Accuracy</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>NU Discovery Cohort</td>
<td>0.92</td>
<td>0.80</td>
<td>0.76</td>
<td>0.84</td>
<td>0.58*</td>
<td>0.93*</td>
</tr>
<tr>
<td>CTOT14 Validation Cohort</td>
<td>0.72</td>
<td>0.81</td>
<td>0.50</td>
<td>0.90</td>
<td>0.58</td>
<td>0.87</td>
</tr>
</tbody>
</table>

*Prevalence-adjusted PPV and NPV
Pre-AR, Pre-ADNR, Pre-TX have different trajectories over time (p<0.001)

High NPV pre-AR, leading to confidence in detecting immune quiescence in serial monitoring

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-AR vs. Pre-TX</td>
<td>0.26</td>
<td>0.75</td>
<td>0.17</td>
<td>0.84</td>
</tr>
<tr>
<td>Pre-AR vs. Pre-nonAR</td>
<td>0.26</td>
<td>0.71</td>
<td>0.13</td>
<td>0.85</td>
</tr>
</tbody>
</table>
Blood vs. Graft Transcripts Pre-Withdrawal

PBMC

Allograft

Bohne et al. JCI 2012
93 probes in biopsy
  • distinguish TOL vs. non-TOL at baseline
  • Minimal similarity between biopsy and blood genes

* A previously identified biopsy gene signature accurately predicted TOL in 12/14 (85.7%)


Levitsky et al. AASLD 2017
Future Directions

• Non-invasive biomarkers of rejection in Liver Transplant
  – Few clinical applications as of yet
  – Most are diagnostic at time of AR

• High need for predictive biomarkers to advance clinical management
  – Prior to acute rejection
  – Prior to IS minimization or full withdrawal
  – Select patients for specific monitoring and interventions, such as augmentation or minimization of immunosuppression

• Need randomized controlled trials testing biomarkers vs. standard of care in managing immunosuppression (optimization) to improve outcomes
THANKS